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Low worldwide genetic diversity in the killer whale (*Orcinus orca*): implications for demographic history

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A low level of genetic variation in mammalian populations where the census population size is relatively large has been attributed to various factors, such as a naturally small effective population size, historical bottlenecks and social behaviour. The killer whale (*Orcinus orca*) is an abundant, highly social species with reduced genetic variation. We find no consistent geographical pattern of global diversity and no mtDNA variation within some regional populations. The regional lack of variation is likely to be due to the strict matrilineal expansion of local populations. The worldwide pattern and paucity of diversity may indicate a historical bottleneck as an additional factor.

Keywords: population bottleneck; population genetics; marine mammals

1. INTRODUCTION

Measures of intraspecific genetic diversity vary considerably across taxa, with a tendency for large mammals to show relatively low levels (see reviews in Nei 1987; Avise 1994). Small effective population size and historical bottlenecks have variously been invoked to explain this observation. A hypothesis to specifically explain low mtDNA variation for matrifocal odontocetes, such as the killer whale (*Orcinus orca*), was based on the 'hitchhiking' of haplotypes with maternally transmitted cultural traits (Whitehead 1998). In this way, selection among matrilines could limit mtDNA diversity over time, but the model of Whitehead (1998) relies on the fairly strict maternal transmission of cultural traits. An alternative theory indicates that matrilineal social structure and heterogeneity in reproductive success among populations in space and time can account for reduced mtDNA diversity (Tiedemann & Milinkovitch 1999). Neither theory necessarily accounts for low variation at nuclear markers, unless male-mediated gene flow is also very much restricted.

The killer whale has a distribution range across all major oceans in polar and temperate waters. Social groups of this species can be highly stable, and regional populations of known individuals persist for decades at least (see Bigg *et al.* 1990). In the eastern North Pacific (ENP), where killer whales have been studied since the 1970s, there are at least five putative populations, defined by their behaviour and geographical distribution. These are known as 'transients' (marine mammal foraging specialists), Alaskan, northern (in British Columbia) and southern (in British Columbian and US waters off Washington State) 'residents' (fish foraging specialists), and 'offshores' (Bigg *et al.* 1990; Dahlheim *et al.* 1997; Ford *et al.* 1998; Ford & Ellis 1999). In the ENP the transient and resident populations have already been shown to be genetically differentiated (Hoelzel & Dover 1991; Hoelzel *et al.* 1998). In other parts of the world local populations are also known to show site fidelity, long-term associations, and prey primarily on marine mammals (e.g. at Punta Norte in Argentina; Lopez & Lopez (1985) and Hoelzel (1991)) or fish (e.g. off Iceland (Sigurjónsson *et al.* 1988), and in the North Sea (Simila *et al.* 1996)). The demographic structure of these putative populations has been described using the following terms. A stable social group is referred to as a 'pod', while a regional group of associating pods is known as a 'community'. We will refer to the latter as a 'population'. We will also identify regional samples about which nothing is known of the pod structure or associations as representing putative populations.

We investigated mtDNA diversity at ATPase6, ATPase8, $tRNA_{pro}$ and control region loci among samples from the ENP, western South Pacific (WSP), western North Atlantic (WNA), eastern North Atlantic (ENA), western South Atlantic (WSA) and Antarctic (ANT). We also report on microsatellite DNA diversity in comparison with other delphinid species, and provide a preliminary assessment of variation among ENP populations. We find a pattern of genetic diversity consistent with a historical bottleneck event, but also indicative of what may be a uniquely strict matrilineal structure within regional populations.

2. MATERIAL AND METHODS

(a) *Sample collection*

Samples were collected from stranded, captive and free-range whales (the latter by biopsy sampling, see Hoelzel *et al.* (1998)).

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Sampling strategy for the Alaskan resident (AR) and southern resident (SR) putative populations (only) included females that represented known, extended matrilines. In this way we could infer the genotype of unsampled individuals as follows. From the AF pod (AR population) the primary females from each of five matrilines were sampled, representing 32 whales from known maternal lineages (based on genealogies determined from observed births). From the AG pod (AR population) four females representing 16 maternal relatives were sampled. From Washington State (SR population), the J pod was sampled for three females representing ten maternal relatives, the K pod for two females representing seven maternal relatives, and the L pod for three females representing 12 maternal relatives. The maternal relationship was unknown for five adult males from AR and two adult males from SR. One further SR male provided a maternal link to an additional four whales. Therefore, from a total of 18 sequences (17 females and one male) we could infer the mitochondrial genotypes of an additional 81 whales. Further details on the sample sizes are indicated in the following paragraphs, but note that all sample sizes indicated reflect only the analysed samples, and not additional individuals for whom mitochondrial genotypes can be inferred. The total sample set also included some pairs of whales known to be related along a matriline, and only one individual from these pairs was sequenced for mtDNA. DNA was extracted by standard phenol–chloroform methods.

The strategy for microsatellite DNA loci was as follows for the interspecific comparisons. An initial sample of up to 49 killer whales that included all geographical regions (and both transients and residents from the ENP) was amplified for all loci. For those loci that showed variation, the sample size was increased to up to 96. These sample sets include samples from stranded animals about which the source population was unknown, and individuals known to be related along a matriline (that were excluded from the mtDNA analyses). Comparisons of putative populations in the ENP included all available samples.

(b) *PCR amplification*

Primers situated in $tRNA_{thr}$ and $tRNA_{phe}$ amplify the $tRNA_{pro}$ and entire control region loci ($n = 102$, including 47 ENP, 45 ENA, five WSA, three WSP, one WNA and one ANT), for a combined total of 995 bp (for primers and reaction conditions see Hoelzel & Green (1998)). No variation was found in the $tRNA_{pro}$ region, and therefore this amplification product is referred to as the 'control region'. *ATPase8* and *ATPase6* genes were amplified from some of the same whales ($n = 33$, including 26 ENP, four ENA, two WSA and one WSP) for a combined total of 817 bp using primers placed in the $tRNA_{lys}$ and *CO3* genes—sense, 5′-AGCATTAACCTTTTAAGTTA; antisense, 5′-GGTTTGGTGGGTCATTA. ATPase loci were included primarily to help resolve the pattern of variation in the ENP, and were amplified in the same reaction conditions as above. Amplified DNA was purified on QIAGEN spin columns and sequenced forward and reverse using the ABI 377 automated system.

Microsatellite DNA was amplified from eight loci including Ev37, Ev92 (Valsecchi & Amos 1996), BA417 (Schlotterer *et al.* 1991), KWM2a, KWM12a (Hoelzel *et al.* (1998); amplification protocol as in this reference), *KWM*1b, *KWM*2b and *KWM*9b. Primer sets for previously unpublished loci: *KWM*1b: 5′-TAA GAACCTAAATTTGGC, 5′-TGTTGGGTCTGATAAATG; *KWM*2b: 5′-AGGGTATAAGTGTTAAGG, 5′-CAACCTTA TTTGGATTTC; *KWM*9b: 5′-TGTCACCAGGCAGGACCC, 5′-GGGAGGGGCATGTTTCTG. The reaction conditions for the latter three loci were as in Hoelzel *et al.* (1998), except for the following: *KWM*1b, annealing at 45 °C; *KWM*2b, annealing at 44 °C; *KWM*9b, annealing at 55 °C; Mg at 1.5 mM for all three loci.

(c) *Data analysis*

Haplotypes were aligned using PILEUP (GCG computer package) and compared using routed and un-routed maximum parsimony phylogeny reconstructions (using PAUP* 4.2). The transition–transversion ratio was set at observed levels. Bootstrap analyses were run for 1000 replications. One outgroup species (bottlenose dolphin, *Tursiops truncatus*) was sequenced using the method described in § 2b, and the other (Risso's dolphin, *Grampus griseus*) was from GenBank (AB018584). The calculation of nucleotide diversity was after Nei (1987) and computed using ARLEQUIN v. 2.0 (Schneider *et al.* 1999). Measures of genetic distance used were the Kimura (1980) two-parameter method for comparisons of mtDNA sequence data, and $\delta\mu^2$ (Goldstein *et al.* 1995) for microsatellite loci. F_{ST} and the significance of its difference from zero were calculated using FSTAT (Goudet 1999).

A mismatch analysis (the distribution of the observed number of differences between pairs of haplotypes) was conducted to investigate demographic history (Rogers & Harpending 1992). Due to the fact that the mtDNA genotypes of multiple samples from within a region were identical, a mismatch analysis including all samples had very high values at zero and at the high end of the distribution. However, we feel that the genetic data are consistent with observational data indicating extended matrilines within some regional populations (e.g. see Bigg *et al.* 1990; Dahlheim *et al.* 1997), in which case the inclusion of multiple samples from these regions would bias the analysis through the inclusion of close kin. Therefore, we included only one haplotype from each regional extended matriline, but included the haplotype more than once when it was found in different geographical regions. Only data for the control region were analysed, as much greater variation was revealed for this locus. Data are compared against a simulation generated by a generalized nonlinear least-square approach, as implemented in ARLEQUIN v. 2.0. Using the same restricted sample set, we assessed the *D* values of Tajima (1989) and the *F* values of Fu (1997) that were estimated using ARLEQUIN v. 2.0.

3. RESULTS

Worldwide mtDNA diversity is low for the killer whale ($\pi = 0.0052 \pm$ s.d. 0.0031 for the control region, $\pi = 0.0039 \pm$ s.d. 0.0026 for the ATPase loci; cf. Hoelzel *et al.* (1998)). A level of diversity this low is most common among species thought to have undergone a bottleneck, and unlike various other cetacean species, there is little correspondence between geographical populations and genetic distance (for a comparative review among marine mammal species see Hoelzel *et al.* (2002)). Phylogenies of the entire control region (figure 1) show the same haplotypes in different oceans and different hemispheres. Similar to the 'resident' populations in the ENP, there were just two haplotypes in Icelandic waters, differing by 1 bp. The most differentiated haplotypes are those representing the ENP transient population and the one sample from the Ross Sea. The transients show a Kimura two-parameter genetic distance (Kimura 1980) of 0.0051–0.0123

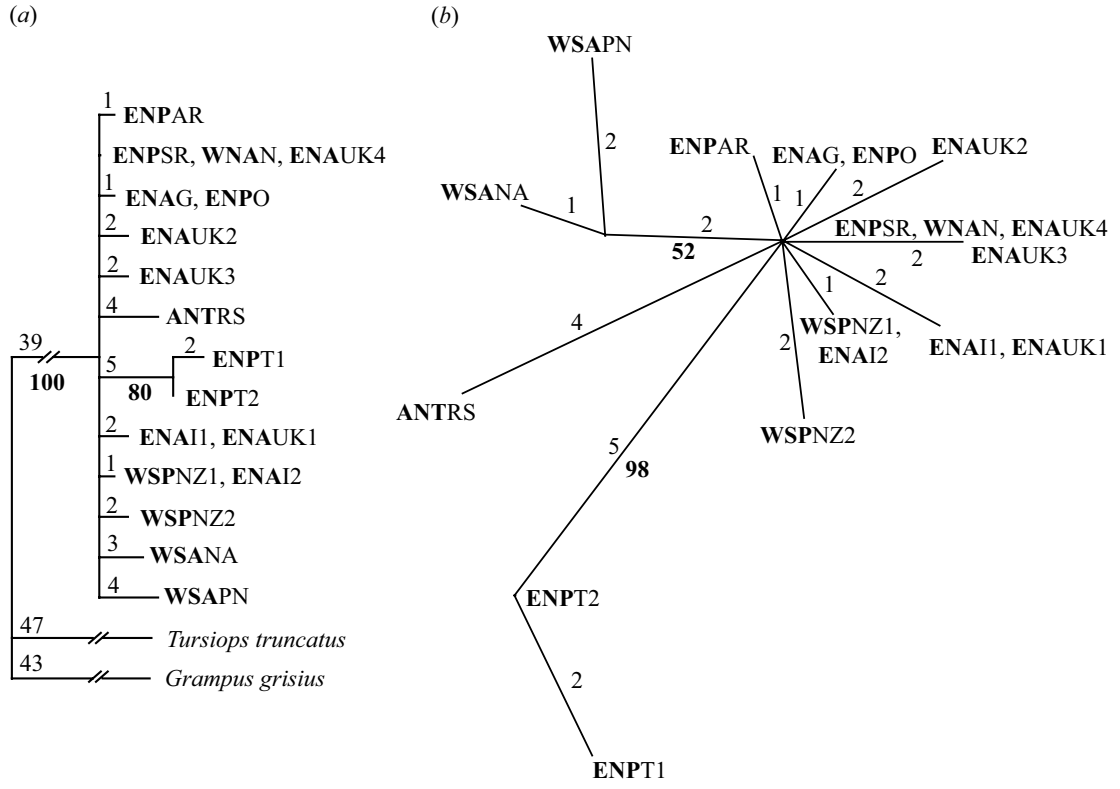

Figure 1. Maximum parsimony phylogenies of control region haplotypes ((*a*) routed, (*b*) unrouted). Bold numbers give bootstrap support (based on 1000 reiterations), branch numbers give the number of steps. Bold lettering indicates the major oceanic regions (see § 1), while the normal type describes the local populations and different haplotypes as follows: PN, Punta Norte, Argentina ($n = 3$); NA, northern Argentina ($n = 2$); AR, Alaskan residents ($n = 15$); SR, southern residents ($n = 11$); N, Newfoundland ($n = 1$); G, Germany ($n = 1$); O, offshores ($n = 6$); NZ1, New Zealand haplotype 1 ($n = 2$); NZ2, New Zealand haplotype 2 ($n = 1$); I1, Iceland haplotype 1 ($n = 16$); I2, Iceland haplotype 2 ($n = 23$); UK1, UK haplotype 1 ($n = 1$); UK2, UK haplotype 2 ($n = 1$); UK3, UK haplotype 3 ($n = 2$); UK4, UK haplotype 4 ($n = 1$); T1, transient haplotype 1 ($n = 14$); T2, transient haplotype 2 ($n = 1$); RS, Ross Sea ($n = 1$).

in pairwise comparison with all other populations, while the Ross Sea haplotype distances range from 0.0045 to 0.0123 (Tamura–Nei distances were identical—data not shown). The genetic distances among all other pairwise comparisons range from 0.0006 to 0.0049. ENP transients show no closer relationship to marine-mammal specialists from other parts of the world (e.g. WSA) than they do to known fish specialists, either in sympatry (ENP residents) or allopatry (e.g. ENA, WSP).

An alignment of the 13 control region haplotypes illustrates two unexpected features (figure 2). In general, cetaceans show less diversity within a central conserved domain of the control region (Hoelzel *et al.* 1991) as seen in other taxa (e.g. Anderson *et al.* 1982). However, 30% of variable sites in the killer whale control region are within this conserved domain (figure 2). This compares with 8.3% among sperm whale (*Physeter macrocephalus*) populations (Lyrholm *et al.* 1996), 11.4% comparing the killer whale with Commerson's dolphin (*Cephalorhynchus commersonii*) and 8.0% comparing the killer whale with the minke whale (*Balaenoptera acutorostrata*; Hoelzel *et al.* 1991). The killer whale transition : transversion ratios also appear relatively low at 3.75 for the control region and 2.00 for the ATPase loci, although these ratios are based on only a few changes.

The alignment is boxed to highlight the ENP transient and Argentine lineages, also reflected in the structure of the phylogeny (figure 1). If variation was restricted by a bottleneck event to haplotypes now represented by lineages reflected in the phylogeny (e.g. four lineages represented by the ENP transients, WSA, ANT and the remainder), most of the variation within the conserved region could be explained by the ENP transient lineage, and most of the transversions could be explained by the WSA lineage (figure 2). Divergence among lineages at the conserved domain (1.6–2.4%) is within the range seen for some other delphinid species (e.g. Mediterranean versus British coastal *T. truncatus* populations: 0.0–5.2%, based on 54 pairwise comparisons; A. Natoli & A. R. Hoelzel, unpublished data). Excluding the ENP transient, WSA and ANT putative lineages (leaving the putative lineage with the largest number of haplotypes), the proportion of variable sites within the conserved domain becomes 9.5%.

We can estimate the time that has elapsed since a putative bottleneck by assessing the accumulated variation within lineages. Considering only transversions, the average number of changes per lineage is 0.67 (range = 0–1; excluding ANT where the sample size is 1). Based on estimates of the transversion mutation rate from interspecific comparisons (Barnes *et al.* 1985; Hoelzel *et al.* 1991), this would indicate a bottleneck event *ca.* 145 000 to 210 000 BP (based on *O. orca* versus *C. commersonii*: 23 transversions; estimated 5 Myr divergence time; and *O. orca* and *C. commersonii* versus *B. acutorostrata*: 95 transversions

control region

	75	135	153	171	217	346	348	353	354	374	* 395	* 462	* 479	* 528	* 573	* 575	* 612	696	866	975	984
ENPAR	A	G	T	A	G	G	T	C	T	A	A	T	A	C	C	T	T	T	A	T	C
ENPSR, WNAN, UKKW4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.
ENPO, ENAG	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	C	.	.	.
UKKW2	.	.	.	.	.	A	C	.	.	.	.	.	.	.	.	C	.	.	.	.	.
ENPT1	-	.	.	.	A	.	.	.	.	.	.	C	G	T	T	C	C	.	G	.	.
ENPT2	-	.	.	.	.	.	.	.	.	.	.	.	G	T	T	C	C	.	G	.	.
UKKW3	.	.	.	.	.	.	.	.	.	T	G	.	.	.	.	C	.	.	.	.	.
ENAI1, ENAUK1	-	.	C	.	.	.	.	.	.	T	.	.	.	.	.	C	.	.	.	.	.
ENAI2, WSP NZ1	-	.	.	.	.	.	.	.	.	T	.	.	.	.	.	C	.	.	.	.	.
WSPNZ2	-	A	.	.	.	.	.	.	.	T	.	.	.	.	.	C	.	.	.	.	.
WSANA	-	.	.	.	.	.	.	.	.	T	.	.	.	.	.	C	.	.	.	G	G
WSAPN	-	.	.	.	.	A	.	.	.	.	.	C	.	.	.	C	.	.	.	G	G
ANTRS	N	.	.	G	.	A	.	T	C	.	.	.	.	.	.	C	.	.	.	.	.

Figure 2. Alignment of the 13 control region haplotypes, generated by PILEUP from the GCG package. Numbers indicate variable sites along the sequence, where '1' begins with the $tRNA_{pro}$ locus, and the control region begins at 74. The central conserved region is indicated by asterisks. Two lineages are indicated by the blocked sequence.

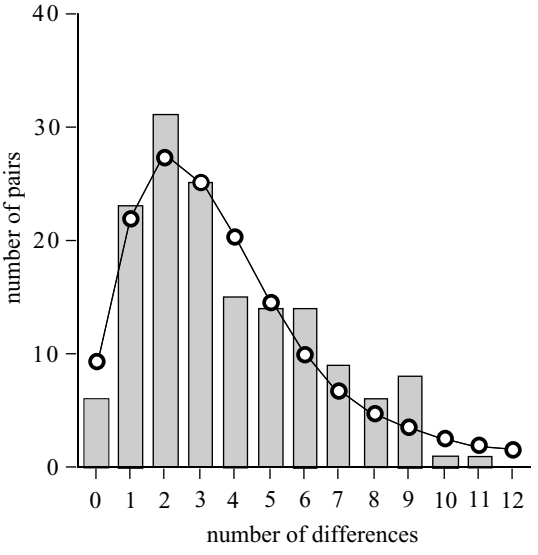

Figure 3. Mismatch distributions for the control region. The observed number of differences are given as bars in the histogram while the simulation for an expanding population is given as a line.

(average); estimated 30 Myr divergence time). However, given the incomplete and uneven representation of haplotypes within lineages and the small number of transversions, this will be an approximate estimate.

An independent assessment of demographic history can be provided by tests for neutrality. Significant negative *D* values of Tajima (1989) and *F* values of Fu (1997) indicate a deviation from the expectations of the mutation–drift equilibrium, and can indicate population expansion (Tajima 1989; Fu 1997), although rate variation along the sequence can inflate *D* (Aris-Brosou & Excoffier 1996). A negative *D* value was found for each locus (control region: $D = -1.36$, ATPase: $D = -0.984$), although neither was significant. The *F* values, however, were negative and significant for each locus (control region: $F = -6.20$, $p = 0.003$; ATPase: -2.62, $p = 0.03$). Mismatch distributions (Rogers & Harpending 1992; Schneider *et al.* 1999) can also provide an indirect assessment of demographic history. A mismatch distribution for the control region locus is shown in figure 3. While a unimodal distribution is expected for expanding populations (indicated by the simulation curve in figure 3), stable populations produce ragged distributions. In this case, the observed distribution closely matches the simulation data, indicating expansion.

Five microsatellite DNA loci isolated from a killer whale genomic library (Hoelzel *et al.* 1998) and two derived from the humpback whale (*Megaptera novaeangliae*; Valsecchi & Amos 1996) were compared for levels of variability among three delphinid species—the killer whale, the bottlenose dolphin and the common dolphin. The killer whale diversity was the lowest (average of 4.1 alleles per locus compared with 11.3 for *T. truncatus* and 8.9 for *D. delphis*), and three of the loci derived from the killer whale were monomorphic for the killer whale, but polymorphic in the other two species (table 1). For all loci the allele size for the killer whale falls within the range for the other species.

For a focal analysis of killer whales sampled in the ENP we compared putative populations of fish specialists in Southeast Alaska (AR) and off Washington State (SR) with sympatric marine-mammal specialists (T), and T samples from California and Mexico. The caption to figure 1 lists the numbers of samples from different ENP populations sequenced for the control region. The ATPase loci were sequenced for 17 transient, four offshore, three AR and two SR whales. Comparisons at the mtDNA loci show just 1 bp difference in 1812 (0.06%) between SR and AR samples. Individuals from all three SR pods (social groups) and all four AR pods showed no variation within regional populations. The most common T haplotype (ENPT1) was the only haplotype found in 13 pods (sequenced for the control region, and for the full 1812 bp in nine of these pods). The combined sequences were identical for this haplotype in different pods sampled from Alaska, Washington State, California and Mexico (Gulf of California).

Four variable microsatellite DNA loci (BA417, KWM2a, KWM12a and Ev37) were compared among the SR, AR and T populations. The measures of genetic distance (table 2) showed significant differentiation for all populations (all F_{ST} values were significantly greater than zero). However, differentiation between the T and AR populations was relatively high, while T and SR were only

Table 1. Number of alleles (allele size range) and observed heterozygosity (Ho) for seven loci in three delphinid species.
(Killer whale sample sets include individuals from the most dissimilar mtDNA lineages (including 'transients' and 'residents'), and from different geographical populations. The bottlenose sample is restricted to those populations known to be *T. truncatus* (no '*aduncus*-type' samples were included). The common dolphin samples include only the 'short-beaked' form.)

	killer whale			bottlenose dolphin			common dolphin		
	n	alleles	Ho	*n*	alleles	Ho	*n*	alleles	Ho
KWM1b	43	1 (192)	0	101	5 (180–196)	0.13	20	3 (186–194)	0.15
KWM2a	83	7 (139–163)	0.72	102	9 (144–158)	0.69	18	10 (149–173)	0.67
KWM2b	49	1 (174)	0	110	8 (166–182)	0.26	11	6 (170–180)	0.75
KWM9b	36	1 (171)	0	98	8 (171–185)	0.54	11	9 (161–193)	1.0
KWM12a	87	9 (167–183)	0.69	100	13 (161–185)	0.69	19	8 (167–181)	0.79
Ev37	96	9 (200–226)	0.59	97	29 (192–252)	0.89	19	20 (176–240)	0.95
Ev92	35	1 (252)	0	27	7 (239–279)	0.63	18	6 (240–256)	0.83

Table 2. Pairwise genetic distance between three putative populations.
(Based on microsatellite DNA variation at four loci: $\delta\mu^2$ (above) and F_{ST} (below). Sample size and standard error are given in parentheses.)

	AR	SR	T
AR (32)	—	3.97 (2.06)	11.19 (5.52)
SR (25)	0.087 (0.017)	—	1.99 (0.083)
T (26)	0.202 (0.024)	0.116 (0.032)	—

about as differentiated as SR and AR. Given the mtDNA and observational data, it seems likely that 'within population' samples will be made up of close relatives. Therefore the magnitude of these distance measures will be biased, but interpretation based on the relative patterns should be informative.

4. DISCUSSION

Local populations of this species are thought to expand by pod fission, and there is no evidence for dispersal of males or females from some natal populations (Bigg *et al.* 1990; Dahlheim *et al.* 1997; Ford *et al.* 1998). This would explain the lack of mtDNA variation among groups of pods within a geographical range. However, local matrilineal expansion may not fully explain the general paucity of genetic variation in this species. The cultural 'hitchhiking' theory of Whitehead (1998) could potentially explain the low mtDNA variation. It could also explain low microsatellite DNA variation if mating was largely within matrilineal groups. However, our data cannot exclude male-mediated gene flow among matrilineal groups (based on the relative pattern of microsatellite distances among the ENP samples, although even if biased by the inclusion of kin, the observed F_{ST} values may be high enough to imply a low rate).

Our sample size was too small for most geographical regions for a conventional analysis of population structure, but wherever a larger number of samples were available (in the ENP and off Iceland), blocks of individuals from the same regional populations showed the same mtDNA haplotype. In the ENP this represented four well studied populations (ARs, SRs, transients and offshores), and in Iceland two less well defined putative populations with overlapping distributions along the southern Icelandic coast. If this were a consistent pattern, the multiple genotypes found off the British Isles may simply reflect the infrequent sighting of this species in those waters, and indicate representation of the overlapping range of populations from other regions (especially Iceland and Norway).

Mitochondrial DNA genotypes from distant geographical regions were in some cases identical. This seems unlikely to represent the same extended matrilines being re-sampled in different locations (or recent dispersal events), because matches were found over such large distances (including matches between the South Pacific and North Atlantic). A more parsimonious explanation is that the distribution of these haplotypes reflects the stochastic distribution of genotypes following a post-bottleneck expansion. This hypothesis is supported by the widest distribution (occurring in the ENP, WNA and ENA) being seen for the haplotype appearing at the centre of the star phylogeny (see figure 1). The most common mtDNA haplotypes were shared between oceans for the sperm whale (which has been suggested to have undergone a bottleneck (Lyrholm *et al.* 1996; Lyrholm & Gyllensten 1998)), but haplotype sharing was less pronounced over a broad geographical range for a mtDNA RFLP study of the humpback whale, and no haplotypes were found in common between the North Atlantic and the ENP for this species (Baker *et al.* 1994). While shared haplotypes over a broad geographical range are not uncommon among cetacean species, each population is typically diverse, including unique haplotypes (for a review see Hoelzel *et al.* 2002). The striking feature of the killer whale pattern for the samples in this study, is that all individuals within a putative population often share the same haplotype, and this may be the same haplotype as seen in other oceans or hemispheres.

Other aspects of the observed mtDNA variation also seem to indicate a bottleneck event. For example, the relatively high variation in the central 'conserved' domain of the control region (indicating the chance post-bottleneck survival of divergent haplotypes), low nucleotide diversity, the characteristic star phylogeny and the evidence indicating expansion (the mismatch distribution and *F* values). The large magnitude of the sequence differentiation seen

between 'transient' and 'resident' haplotypes in the ENP (and see Hoelzel *et al.* 1998) may therefore simply reflect a historical accident following a post-bottleneck expansion.

A selective sweep on a mtDNA gene or a process such as the cultural hitchhiking proposed by Whitehead (1998), are possible alternative interpretations for the data indicating a deviation from the mutation–drift equilibrium. However, an analysis of variation at microsatellite DNA loci provided an independent indication of low genetic diversity in the killer whale (supported by earlier work on minisatellite DNA variation (Hoelzel & Dover 1991)), consistent with a bottleneck event. While not a definitive assessment, the comparison among delphinid species, especially with respect to the five loci derived from the killer whale, indicates diminished microsatellite DNA variation in the killer whale. Four of these loci (KWM1b, KWM2a, KWM9b and KWM12a) have been screened for five delphinid species in our laboratory, and the average number of alleles at these loci for the killer whale (4.5) is less than that for any of the other four species (*T. truncates*, 8.8; *D. delphinis*, 7.5 (from table 1), and striped dolphin *Stenella coeruleoalba*, 13.0; *G. griseus*, 8.0 (S. Gaspari, personal communication)). Among the loci variable for the killer whale (from table 1), the average number of alleles is 8.3 for the killer whale, but 17 for *T. truncates* and 12.7 for *D. delphis*. These differences cannot be easily explained by sampling, as the sample size and geographical spread are in most cases highest for the killer whale.

Mutation rates for mammalian microsatellite loci have been estimated to range from 10^{-5} to 10^{-3} per locus per generation (Dallas 1992; Weber & Wong 1993; Ellegren 1995). The lower of these rates would be consistent with monomorphic loci 10^5 years after variation was lost. It is also consistent that loci fixed in the killer whale are the least variable loci in the other species (table 1), although other factors, such as differential rates of turnover, could be involved in determining the variability of these microsatellite loci (e.g. see Hancock 1999).

If there was a bottleneck event, it may have occurred at one of the glacial terminations, such as the unusually extreme event at the Eemian termination, *ca.* 130 000–140 000 BP (Petit *et al.* 1999), which is consistent with our rough estimate of the timing of a putative bottleneck event. Rapid climatic shifts may have been associated with switches in oceanic thermohaline circulation, at least in the North Atlantic, possibly occurring over a period of decades (e.g. Weaver & Hughes 1994), which could in turn have an impact on the distribution and abundance of killer whale prey. Killer whales tend to be resource specialists, and may require considerable investment in learning the temporal and spatial distribution of prey, in addition to techniques for efficient capture (e.g. see Hoelzel 1991; Guinet 1992; Hoelzel 1993; Baird & Dill 1996). This could make relatively rapid transitions difficult to adjust to.

While a bottleneck may have reduced genetic variation in this species, social behaviour and in particular an unusual pattern of strict maternal philopatry has led to highly structured genotypic variation at the population level. Our strategy of sampling individuals who represented extended matrilines in the ENP further supports the conclusion that there is no mtDNA variation within these putative populations, at least for the sequence analysed. This is consistent with observational data for some populations indicating no female dispersal over the course of studies lasting up to 30 years (e.g. Bigg *et al.* 1990; Dahlheim *et al.* 1997). The result is extended regional 'clans' that can be represented by hundreds of individuals (or possibly much fewer in some cases). This pattern of population expansion could clearly lead to reduced global haplotypic diversity. However, other factors (as discussed in the previous paragraph) indicate a population bottleneck as an additional factor explaining low diversity in this species.

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